EFFECT OF RUMEN-PROTECTED METHIONINE AND / OR LYSINE SUPPLEMENTATION TO THE RATIONS ON PRODUCTIVE PERFORMANCE OF LACTATING BUFFALOES

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(Received 6/11/2005, accepted 15/12/2005)

SUMMARY

Twelve lactating buffaloes were divided to four groups (three animals each) using 4 x 4 Latin square design. Animals were fed on control diet, which consisted of concentrate feed mixture : berseem clover : rice straw (70: 15: 15, dry matter basis). Rumen protected amino acids were added to the control diet either at 15 g Smartamine ^{TM}M (protected methionine), (Me), 40 g protected Lysine (Ly), or 15 g Smartamine + 40 g protected lysine (Me+Ly) for 4 months during lactation. Milk yield, 4% FCM (9.30, 11.41, 10.52 and 12.34 kg/h/d for control, Me, Ly and Me+Ly groups, respectively). Fat percent and yield, total solids percent and yield; total protein percent, casein percent (2.80, 3.07, 3.02 and 3.28% for control, Me, Ly and Me+Ly groups, respectively) and lactose percent significantly ($P<0.01$) increased with treated groups than those of the control. Also, milk non-protein-nitrogen percent decreased ($P<0.01$) with treated groups than those of the control (0.042, 0.029, 0.036 and 0.027% for control, Me, Ly and Me+Ly groups, respectively). Blood serum total protein and serum glucose were increased ($P<0.05$) in treated groups, while, serum albumin, serum globulin and serum total lipids were increased ($P<0.05$) with (Me+Ly) and (Me) treatments compared with the control.

The observed data might suggest that methionine addition (alone or in combination) was more effective than lysine. It could therefore be concluded that methionine was probably the first limiting amino acid under the present conditions. In conclusion, rumen protected methionine and/or lysine supplementation to rations of lactating buffaloes had beneficial effects on milk yield and composition without any adverse effect on the animal health.

Keywords: *methionine, lysine, buffaloes, blood, milk*

INTRODUCTION

The amino acid requirement is significantly affected by the rumen degradability of dietary crude protein. The differences in degradation of crude protein from forages and concentrates and the dynamics of microbial protein synthesis in the rumen have made it difficult to significantly alter the ratio and the quality of amino acids reaching the small intestine (Seymour *et al* 1990 and Schwab *et al* 1992). Previous studies support the hypothesis that the principal sources of variation in the amino acid balance of intestinal digesta are the amino acid composition of feed proteins and the proportional flow of ruminally undegraded intake protein and microbial protein to the intestines (Rulquin *et al* 1995). Attempts to increase the passage of essential amino acids to the intestines by feeding protein sources with low rumen degradabilities had variable success (Seymour *et al* 1990). Although it is still not common to consider individual amino acids when formulating diets for lactating animals, there is increasing evidence that the addition of certain amino acids can improve milk production and particularly milk protein content. Two amino acids, methionine and lysine, are of special interest for dairy cows as have been identified as the most limiting amino acids for the synthesis of milk protein (Schwab *et al* 1992; Rulquin and Verite, 1993; Guinard and Rulquin, 1995; and Rulquin *et al* 1995). Unfortunately, free crystalline methionine and lysine is easily and rapidly degraded by rumen bacteria and a variety of approaches have been used to achieve delivery of methionine or lysine to absorption sites. Protection of methionine has been attempted by formation of amino acid analogues or derivatives (Patterson and Kung, 1988) or by encapsulation (Overton *et al* 1996). Lysine protection has been attempted by using formaldehyde treatment (Aly *et al.* 2005).

In this study, the diet of early-lactation buffaloes was supplemented with rumen protected methionine (Smartamine^{TM}) and/or protected lysine in order to study the effects of these supplementations on milk yield, milk composition and some blood serum parameters.

MATERIALS AND METHODS

This study was conducted at the Experimental Farm in Shalakan, Faculty of Agriculture, Ain Shams University and Dairy Science Department, National Research Center, Dokki, Giza, Egypt, during 2004-2005.

1- Animals and rations:

A total number of 12 lactating buffaloes aged 3-7 years were used in the present study. The animals were divided into four groups (three animals each) according to milk yield and animal weight, using 4 x 4 Latin square design. The experimental period was extended for four months (one month each period).

Dietary treatments were (1) control, (2) control + 15g Smartamine^{TM} M (Me) (Protected Methionine, RHONE-POULENC product, France), (3) control + 40g protected lysine (Ly) (Lysine amino acid product of ADWIA company, Egypt), which was treated with formaldehyde 40% according to the method suggested by (Fergueson, 1975), and (4) control + 15 g Smartamine^{TM} M+ 40g protected lysine (Me+Ly). The control ration consisted of concentrate feed mixture (CFM): berseem (B): Rice straw (RS); (70: 15: 15) on dry matter basis. Chemical composition of feed ingredients is shown in Table (1).

2- Management:

Amounts of daily feeds were assessed to cover the maintenance and the production requirements (Shehata, 1971). The CFM was individually weighed for each animal and offered twice daily during milking times at 5.00 and 16.00 hr, while roughages were offered at 9.00 and 11.00 h. after accessing the animals to fresh water. The daily supplemental rumen protected amino acids were daily mixed with CFM just before morning feeding to ensure that each animal had consumed its own supplement.

Method used for lysine protection was as follows: Lysine was sprayed with formaldehyde (40%) calculated to provide 1 gm formaldehyde (HCHO)/ 100gm crude protein (Fergueson, 1975). The treated lysine was stored for 7 days in plastic bags at room temperature before being used.

3- Analysis of feed samples:

Samples of CFM, RS, protected amino acids and feces were analyzed for dry matter (DM), ash, crude protein (CP), crude fiber (CF), and ether extract (EE) according to A.O.A.C. (1995). Nitrogen free extract (NFE) was calculated by difference.

4- Sampling and analysis of milk:

The animals were hand-milked twice daily. Milk yield was recorded daily during the experimental period which extended for 120 days. During the last three days of each period, (30 days) samples of milk were collected from each animal at morning and evening milking. Composite daily milk samples (relative to the quantity of milk produced) were taken from the two milking to determine the total solids, fat, total protein (TP), pH, acidity and ash (Ling, 1963), lactose (Barnett and Abd El-Tawab, 1957), protein fraction (Aschaffenburg and Drewry, 1959).

5- Sampling and analysis of blood serum:

The blood samples were taken at the final day of milk sampling at four hours after morning feeding from the jugular vein from all animals. Collected blood samples were centrifuged at 4000r.p.m. for 20 min. and stored in clean glass vials at -18°c till analysis. Serum total protein was determined according to Armstrong and Carr (1964), albumin (Doumas *et al.*, 1971), urea (Patton and Crouch, 1977), transaminases (GOT and GPT) activities (Reitman and Frankel 1957), glucose (Siest *et al* 1981), creatinine (Husdan, 1968), and total lipids (Postma and Stroes, 1968).

6- Statistical analysis:

The data of milk yield, milk composition and blood serum parameters were analyzed according to Statistical Analysis System (SAS, 1998) using Latin Square design where the model was:

$$Y_{ijk} = \mu + T_i + P_j + A_k + E_{ijk}$$

As, Y: expressed every observation of the K^{th} animal in the J^{th} period given I^{th} treatment, T: expressed the treatment effect, P: expressed the periods effect, A: expressed the animal's effect and E: expressed the experimental error. Duncan multiple range test (Duncan, 1955) was carried out for separation among means.

RESULTS AND DISCUSSION

1- Milk yield and composition:

Effect of rumen protected methionine and/or lysine supplementations on milk yield and its composition of lactating buffaloes are shown in Table (2). Milk components yield are shown in Table (3).

Milk yield was significantly (P<0.01) increased by including rumen-protected amino acids in the ration. In other words, Me+Ly, Me and Ly treatments produced 25.9, 17.6 and 11.3% more milk, respectively, as compared to control. These results are in a good agreement with those obtained by Nichols *et al* (1998), Xu *et al* (1998), Iwanska *et al* (1999), Younge *et al* (2001), Noftsger and St-Pierre (2003) who observed that milk production increased with rumen protected methionine and/or lysine supplementation.

The increase in milk yield may be due to one or more of the following reason: 1) Higher dry matter intake, nutrients digestibility and TVFA's content in rumen of animals feed on diets with rumen protected methionine and/or lysine (Aly *et al*, 2005), 2) Apparent increase in the efficiency of nitrogen utilization as well as increased conversion and availability of nutrients necessary for milk synthesis (Iwanska, 1999), 3) Methionine and lysine appear to be most limiting for milk synthesis because they are both heavily utilized by the mammary gland and are present in relatively low concentration in plasma (Clark, 1975).

Yield of 4% fat-corrected-milk (FCM) was significantly (P<0.01) improved by 32.7, 22.7 and 13.1% for Me+Ly, Me and Ly, respectively, compared with control.

Table (1) : Chemical composition of concentrate feed mixture (CFM), berseem clover (B), rice straw (RS), samartamine[tm] M (Me) and protected lysine (Ly) (% Dry matter basis).

Items	Diet ingredients				
	CFM*	B	RS	Me	Ly
Dry matter	91.29	13.3	92.85	95.93	94.60
Organic matter	89.89	88.2	84.55	96.55	97.69
Ash	10.11	11.80	15.45	3.45	2.31
Crude protein	14.15	14.2	3.5	-	-
Methionine	-	-	-	68.23**	-
Lysine	-	-	-	-	63.61[ϕ]
Ether extract	4.05	2.6	2.10	8.60	4.10
Crude fiber	15.33	27.5	33.9	0	3.30
Nitrogen-free-extract	56.36	43.9	45.05	19.72	26.68

* The CFM consisted of 25% undecorticated cotton seed meal, 35% wheat bran, 30% corn, 3% rice bran 3% molasses, 2% limestone, 1% urea and 1% salt (NaCl).
** Total nitrogen is multiplied by (11.1).
[ϕ] Total nitrogen is multiplied by (5.26).

Table (2): Effect of rumen protected AA supplements on overall mean of milk yield and composition of lactating buffaloes.

Items	Treatments				
	Control	Me	Ly	Me+Ly	±S.E
Milk yield (kg/d)	6.65^{D}	7.82^{B}	7.40^{C}	8.37^{A}	0.129
Fat-corrected-milk (kg/d)	9.30^{D}	11.41^{B}	10.52^{C}	12.34^{A}	0.179
Fat content (%)	6.68^{D}	7.01^{B}	6.85^{C}	7.18^{A}	0.035
Total solids content (%)	15.89^{D}	16.37^{B}	16.12^{C}	16.93^{A}	0.072
Solids – not – fat content (%)	9.21^{B}	9.35^{B}	9.31^{B}	9.75^{A}	0.064
Total proteins content (%)	3.72^{D}	4.00^{B}	3.91^{C}	4.17^{A}	0.018
Lactose content (%)	4.66^{C}	4.77^{B}	4.74^{B}	4.95^{A}	0.013
Ash content (%)	0.72	0.72	0.71	0.72	0.010
pH value	6.68	6.68	6.72	6.67	0.011
Acidity	0.17	0.17	0.16	0.17	0.002

Each value of means was obtained from 36 values (12 animals during 3 days).
Means with different superscripts in the same row differ significantly ($P<0.01$).
C= control diet, Me = C + protected methionine, Ly = C+ protected lysine, Me+Ly= C+ protected methionine + protected lysine

These results are in accordance with those reported by Overton *et al* (1998), Nichols *et al* (1998), Bharadwaj and Sengupta (1999) and Iwanska, *et al* (1999). While, Bertrand *et al* (1998); Lui *et al* (2000) and Sancanari, *et al* (2001) reported that rumen protected methionine and/or lysine supplemented ration had no effect on FCM yield. Such discrepancy might be due to the source of dietary protein and its degradability in the rumen and/or to productivity of the animal.

Milk fat percent was significantly increased (P<0.01) with different rumen protected amino acid treatments by 7.5, 4.9 and 2.5%, respectively, for Me + Ly, Me and Ly compared with control. The increase in milk fat may be due to one or more of the following reasons: 1) higher blood serum total lipids concentration (Table, 5) with treated buffalo groups, 2). Methionine in particular might facilitate the transfer of blood lipids to milk by furnishing methyl groups for synthesis of choline and phosphatidylcholine, which represent an important link between methionine and lipid metabolism in ruminants as demonstrated by Seymour *et al* (1990), 3) Methionine might be important for synthesis of lipoproteins and that methionine and lysine facilitated the hepatic secretion of lipoprotein rich in triacyl glycerol as demonstrated experimentally by McCarthy *et al* (1968) and Durand *et al* (1992), 4) Higher dry matter intake in treated groups (Aly et al., 2005). It is possible that similar mechanisms of methionine and lysine action in the mammary gland may be responsible for the increase of milk fat content. The results of this study confirm the data of Xu *et al* (1998), Vanhatalo *et al* (1999), Iwanska *et al* (1999) and Sancanari *et al* (2001). On the other hand, Soder and Holden (1999), Younge *et al* (2001) and Kholif and Kholif (2003) showed that milk fat percent was not affected by rumen-protected amino acids with cows and goats, respectively. Species differences might be involved.

Milk fat yield (Table, 3) was significantly increased (P<0.01) by 35.4, 23.5 and 13.8% for Me+Ly Me and Ly, respectively, compared with control. These results are in good agreement with the findings of Nichols *et al* (1998) and Iwanska *et al* (1999).

Milk total solids (TS) percent were significantly increased (P<0.01) with different supplemented group by 6.5, 3.0 and 1.4%, respectively for Me+Ly , Me and Ly treatments compared with control; similar results of rumen protected protein supplementation, were obtained by Bahardwaj and Sengupta (1999). Also, Me + Ly, Me and Ly treatments produced 34.2, 21.2 and 13.2% more milk TS yield, respectively, as compared to control.

Milk solids not fat (SNF) percent was significantly increased (P<0.01) with Me+Ly treatment, while Me or Ly treatments were did not significantly increase (P>0.05) compared with the control. In other words, Me+ Ly, Me and Ly, respectively, produced 5.9, 1.5 and 1.1% more milk SNF present compared with control. Similar results were obtained by Kholif and Kholif (2003) who found insignificant (P>0.05) increases in SNF with protected methionine. With regard to milk SNF yield, all treated groups significantly increased (P<0.01) SNF yield by 33.3, 19.6 and 12.7% for Me+Ly, Me and Ly, respectively, compared with the control.

Milk total proteins percent were significantly (P<0.01) higher with Me+Ly, Me and Ly treatments by 12.1, 7.5 and 5.1%, respectively, compared with the control. The increase in milk total proteins may be due to one or more of the following reasons: 1) The milk total proteins response to post-ruminal supply of limiting amino acids was much lower on low protein (≤ 14% CP)

Table (3): Effect of rumen protected AA supplements on overall mean of milk component yield (g/day) of lactating buffaloes.

Items	Treatments				
	Control	Me	Ly	Me+Ly	±S.E
Fat yield	443^{D}	547^{B}	504^{C}	600^{A}	8.9
Total solids yield	1055^{D}	1279^{B}	1194^{C}	1416^{A}	21.0
Solids – not – fat yield	612^{D}	732^{B}	690^{C}	816^{A}	12.7
Total proteins yield	247^{D}	313^{B}	290^{C}	349^{A}	5.1
Lactose yield	311^{C}	366^{B}	352^{B}	415^{A}	8.6
Ash yield	47^{C}	56^{B}	53^{B}	61^{A}	1.2

Each value of means was obtained from 36 values (12 animals during 3 days).
Means with different superscripts in the same row differ significantly (P<0.01).
C= control diet. Me = C + protected methionine, Ly = C+ protected lysine, Me+Ly= C+ protected methionine + protected lysine

Table (4): Effect of rumen protected AA supplements on overall mean of milk protein fractions content (%) and yield (g/h/d) of lactating buffaloes.

Items	Treatments				
	Control	Me	Ly	Me+Ly	±S.E
Casein content (%)	2.80^{D}	3.07^{B}	3.02^{C}	3.28^{A}	0.017
Whey protein content (%)	0.825	0.824	0.790	0.811	0.013
Non-protein-nitrogen content (%)	0.042^{A}	0.029^{C}	0.036^{B}	0.027^{C}	0.001
Casein yield	186.58^{D}	240.41^{B}	223.25^{C}	275.41^{A}	3.90
Whey protein yield	54.75^{B}	64.33^{A}	58.66^{B}	67.83^{A}	1.65
Non-protein-nitrogen yield	2.75^{A}	2.25^{B}	2.61^{A}	2.25^{B}	0.093

Each value of means was obtained from 36 values (12 animals during 3 days).
Means with different superscripts in the same row differ significantly (P<0.01).
C= control diet, Me = C + protected methionine, Ly = C+ protected lysine, Me+Ly= C+ protected methionine + protected lysine

Table (5): Effect of rumen protected AA supplements on overall mean of some blood serum parameters of lactating buffaloes.

Parameter	Treatments				±SE
	C	Me	Ly	Me + Ly	
Total protein (g/dl)	6.90^{D}	7.48^{B}	7.18^{C}	7.85^{A}	0.076
Albumin (g/dl)	3.47^{C}	3.67^{B}	3.60^{BC}	3.85^{A}	0.057
Globulin (g/dl)	3.42^{C}	3.80^{AB}	3.58^{BC}	3.99^{A}	0.086
A/G ratio	1.02	0.97	1.01	0.96	0.034
Urea (mg/dl)	42.10	41.14	40.33	40.65	0.760
Creatinine (mg/dl)	1.20	1.20	1.17	1.19	0.064
GOT (units/L)	34.08	33.33	33.58	33.16	1.31
GPT (units/L)	15.66	15.50	16.00	16.00	0.57
Glucose (mg/dl)	56.45^{D}	64.83^{B}	63.15^{C}	67.89^{A}	0.34
Total lipids (mg/dl)	280.50^{b}	294.66^{a}	281.91^{b}	293.00^{a}	4.27

Each value of means was obtained from 36 values (12 animals during 3 days).
Means with different superscripts in the same row differ significantly (P<0.01).
C= control diet, Me = C + protected methionine, Ly = C+ protected lysine, Me+Ly= C+ protected methionine + protected lysine

compared with high protein rations, (Rulquin and Verite, 1993), 2) Methionine must contribute 5.3 to 5.6% of the total essential amino acids in duodenal digesta and 2.5 – 2.7% of the total amino acids, respectively for maximum content and yield of milk protein (Rulquin *et al*, 1995 and Schwab, 1996), 3) Higher CP and OM digestibilities and higher ruminal true protein nitrogen when Baladi goats were fed on diets with rumen protected methionine and/or lysine supplemented (Aly *et al.*, 2005). These results are in a good agreement with those obtained by, Iwanska *et al* (1999); Lui *et al* (2000), Younge *et al* (2001); Rulquin *et al* (2001), Kholif and Kholif (2003) and Noftsger *et al* (2005). The Me +Ly Me and Ly treatments significantly (P<0.01) increased milk total proteins yield by 41.3, 26.7 and 17.4%, respectively, compared with the control. These results are in good agreement with those obtained by Younge *et al* (2001) and Pisulewski *et al* (2002).

Milk lactose percent increased (P<0.01) with Me + Ly, Me and Ly treatments by 6.2, 2.4 and 1.7%, respectively, compared with the control. These results may be due to a significant (P<0.01) increase in blood serum glucose with treated groups (Table, 5). Similar results were reported by Sevi *et al* (1998), who found that milk lactose percent increased when rumen protected methionine and lysine were supplemented to dairy ewes ration. While, Lui *et al* (2000) and Pisulewski *et al* (2002) noted that milk lactose percent was not affected by rumen protected methionine treatment. Data of treated groups show significant (P<0.01) more milk lactose yield by 33.4, 17.7 and 13.2% for Me+Ly, Me and Ly treatments, respectively, compared with control. These results are in accordance with those noted by Sevi *et al* (1998) and Fahey *et al* (2002).

Milk ash percent was not significantly affected (P>0.05) by different experimental treatments. Similar trend of milk ash content was obtained by Kholif and Kholif (2003). Concerning overall means of milk ash yield, data indicate that all treated animal groups increased (P<0.01) milk ash yield by 29.8, 19.1 and 12.8% for Me+Ly, Me and Ly, respectively, compared with control.

Milk pH value and acidity were not significantly (P>0.05) affected by dietary treatments (Table,2).

2- Milk protein fraction:

The data in Table (4) represents the effect of supplementing lactating buffaloes diets with rumen protected methionine and/or lysine on the milk protein fraction. Milk casein content was significantly (P<0.01) higher with Me+Ly, Me and Ly treatments by 17.1, 9.6 and 7.9%, respectively, than control diet. The increase in milk casein may be due to rumen protected methionine or lysine supplementation, which produce 100% of the casein response in early lactation and 47 to 58% of the casein response in peak to med lactation (Schwab *et al* 1992). Similar results were obtained by Blauwiekel *et al* (1997), Bertrand *et al* (1998), Nichols *et al* (1998), Overton *et al.* (1998) and Younge *et al* (2001). Milk casein yield was significantly increased (P<0.01) by 47.1, 28.3 and 19.3%, respectively, for Me+Ly, Me and Ly compared with control. These results are in a good agreement with those obtained by Nichols, *et al* (1998).

Milk whey protein percent was not significantly (P>0.05) affected by different treatments, similar results were obtained by Younge, *et al* (2001) and Pisulewski *et al* (2002) who found that milk whey protein percent was not affected by rumen protected methionine or/and lysine supplemented to dairy animals ration. While Nichols *et al* (1998) reported an increases (P<0.05) in whey protein percent with rumen protected methionine plus lysine. The overall means of milk whey protein yield increased (P<0.01) with Me and Me+Ly by 16.4 and 23.6%, respectively, while, Ly treatment insignificantly increased by 7.3% compared with control. Similar results were obtained by Varvikko *et al* (1999).

Milk non-protein-nitrogen (NPN) percent in all treated buffaloes groups were decreased (P<0.01) by 35.7, 31.0 and 14.3% for Me+Ly, Me and Ly, respectively, compared with control group. These results did not agree with the findings of Varvikko *et al* (1999) and Younge *et al* (2001) who reported that milk NPN percent was not affected by rumen protected methionine or lysine supplemented ration. Values of milk NPN yield were significantly decreased with Me+Ly and Me (P<0.01) by 18.2% than control group, while, Ly treatment insignificantly decreased NPN yield by 5.0% compared with control group.

3- Blood serum parameters:

The data in Table (5) indicate that all treated animal groups had higher (P<0.01) serum total proteins, albumin, globulin and glucose than control. The increases of serum total proteins may be due to the increases in the levels of methionine and lysine in the blood, suggesting that rumen protected methionine and lysine were delivered post-ruminally for absorption from the small intestine. These results are in a good agreement with the findings of Blum *et al* (1999) and Vanhatalo *et al* (1999) who reported that rumen protected amino acids supplementation increased plasma amino acids concentration.

The increases of glucose levels with treated groups agree with those obtained by KröBer *et al*. (2000). This may be due to the increase in TVFA's, whereas, the increase in blood glucose level correlates with an increase in propionic acid level in the rumen (being the main precursor of gluconeogenesis), (Demeterova *et al.* 2002). The higher serum total lipids observed with protected amino acids treatments may be due to methionine and lysine facilitated the hepatic secretion of lipoproteins rich in triacylglcerol (McCarthy *et al*., 1968, and Durand *et al.* 1992). Serum A/G ratio, urea, creatinine, GOT and GPT were not affected by the different experimental treatments. The present values of GOT and GPT showed normal activity of the animal hepatic tissues and the results of GOT and GPT concentration were in the normal range. These results are in accordance with Xu *et al*. (1998). Also, the creatinine content did not significantly differ among treatments. These results indicated to the normal activity of the kidney. KröBer *et al* (2000) obtained similar finding. The results of blood serum urea concentration are in accordance with Pacheco-Rios *et al* (1999), Varvikko *et al* (1999).

Protected methionine and lysine additions insignificantly (P>0.05) reduced the urea contents in serum and especially in milk (Tables 4 and 5). This is a very clear indicator of a relief from protein deficiency at the site of the mammary gland due to an improved amino acid profile.

CONCLUSION

The observed data might suggest that methionine addition (alone or in combination) was more effective than lysine. It could therefore be concluded that methionine was probably the first limiting amino acid under the present conditions. Also, supplementing lactating buffalo diets with rumen protected methionine and/or lysine is recommended as a step in the field of animal production for improving productive performances of lactating buffaloes, regarding milk yield and composition. Additionally, these results revealed that there were no negative effects on general health of the treated animals.

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تأثير إضافة المثيونين و/أو الليسين المحميان من الهدم فى الكرش إلى العليقة على الأداء الإنتاجى للجاموس الحلاب

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استخدمت فى هذه الدراسة 12 جاموسة حلابة حيث قسمت إلى مجموعات تحتوى كل مجموعة على 3 حيوانات وتمت تغذيتها على العلائق التالية:

1- عليقة المقارنة: وتحتوى على 70% علف مركز + 30% علف خشن (15% برسيم + 15% قش أرز) على أساس المادة الجافة.

2- عليقة مضاف إليها الميثونين المحمى من الهدم فى الكرش: عليقة المقارنة مضافا إليها 15 جرام من الميثونين المحمى سمارتامين).

3- عليقة مضاف إليها الليسين المحمى من الهدم فى الكرش: عليقة المقارنة مضافاً إليها 40 جرام من الليسين المحمى.

4- عليقة مضاف إليها الميثونين والليسين المحميان من الهدم معا: عليقة المقارنة مضاف إليها 15 جرام ميثونين محمى + 40 جرام ليسين محمى.

ولقد بدأت التجربة بعد الولادة واستمرت لمدة 120 يوم على 4 مراحل بنظام المربع اللاتينى (4 × 4) كل مرحلة 30 يوم، وقد تم تقييم إنتاج اللبن وتركيبه خلال هذه الفترة، وكان يتم تجميع عينات اللبن آخر 3 أيام من كل فترة، وعينات الدم آخر يوم من كل فترة بعد 4 ساعات من التغذية لجميع الحيوانات، و كانت النتائج المتحصل عليها:

- أدت إضافة الميثونين والليسين المحميان من الهدم فى الكرش معا لعليقة الجاموس الحلاب إلى زيادة معنوية (على مستوى 1%) فى إنتاج اللبن ونسب مكونات اللبن الداخلة وأيضا كمياتها عن المعاملات الأخرى ، كما أدت زيادة معنوية (على مستوى 1%) فى محتوى سيرم الدم من البروتين الكلى، والألبيومين والجلوبيولين والجلوكوز والليبيدات الكلية عن مجموعة المقارنة وحققت أفضل النتائج بالمقارنة بالمعاملات الأخرى.
- أدت إضافة الميثونين المحمى من الهدم وحده فقط للعليقة إلى زيادة معنوية (على مستوى 1%) فى إنتاج اللبن واللبن المعدل نسبة الدهن ونسب مكونات اللبن الداخلية وأيضا كمياتها عن المجموعة المضاف إليها الليسين المحمى ومجموعة الكونترول، كما أدت إلى زيادة معنوية (على مستوى 1%) فى محتوى سيرم الدم من البروتين الكلى والألبيومين والجلوبيولين والجلوكوز والليبيدات الكلية عن مجموعة المقارنة.
- أدت إضافة الليسين المحمى من الهدم وحده فقط للعليقة إلى زيادة معنوية على (مستوى 1%) فى إنتاج اللبن ونسب مكونات اللبن الداخلية وأيضا كمياتها عن مجموعة المقارنة، كما أدت إلى زيادة معنوية (على نسبة 1%) فى محتوى سيرم الدم من البروتين الكلى والجلوكوز فقط عن مجموعة المقارنة.
- تم الحصول على أعلى قيم لإنتاج اللبن واللبن المعدل لنسبة الدهن (4%) ونسب مكونات اللبن الداخلية (الدهن، البروتين، اللاكتوز) وأيضا كمياتها وكذلك محتوى سيرم الدم من البروتين الكلى والألبيومين والجلوبيولين والجلوكوز والليبيدات الكلية فى المجموعة المضاف إلى عليقتها الميثيونين و الليسين المحميان معاً تليها مجموعة الميثونين المحمى فقط ثم تليها مجموعة الليسين المحمى فقط وأخيرا عليقة المقارنة.

فى النهاية يتضح أن إضافة المثيونين و الليسين المحميان من الهدم فى الكرش للحيوانات الحلابة يمكن أن يزيد من إنتاج اللبن ويحسن من مكوناته الداخلية وخاصة بروتين الكازين الذى يعتبر مهما جدا لصناعة الجبن وذلك دون التأثير على صحة الحيوان العامة. كما لوحظ من النتائج أن إضافة المثيونين (منفردا أو مخلوطا) كان أكثر تأثيرا من الليسين فقط، لذلك يمكن إستخلاص أن المثيونين هو الحمض الأمينى الأول المحدد لإنتاج اللبن تحت ظروف هذه الدراسة.